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## **Inter- and transgenerational inheritance of behavioral phenotypes**

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**Abstract:** Adult animal behaviors are determined by complex and dynamic changes in gene expression in different brain regions and are influenced by life experiences and environmental exposures. These stimuli affect gene expression through intricate mechanisms of regulation that largely implicate epigenetic factors, such as, DNA methylation, histone post-translational modifications, and non-coding RNAs (ncRNAs). Through these molecular pathways, some of the behavioral phenotypes associated with life experiences can be stably transmitted to descendants, sometimes across several generations. Rodent studies indicate that parental stressful and traumatic experiences can lead to behavioral despair, risk-taking behaviors, altered sociability and atypical responses to stressful stimuli in the offspring, whereas parental environmental enrichment has been associated with improved cognition and stress resilience in the offspring. Similar observations have been made in humans; children and grandchildren of genocide survivors show increased psychopathology and emotional disturbances. At the molecular level, changes in germline ncRNAs have been identified as likely vectors of transmission in rodents. The mechanisms linking behavioral stimuli to the germline, and factors responsible for these changes and their persistence across generations remain, however, largely unidentified.

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# Inter- and transgenerational inheritance of behavioral phenotypes

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Adult animal behaviors are determined by complex and dynamic changes in gene expression in different brain regions and are influenced by life experiences and environmental exposures. These stimuli affect gene expression through intricate mechanisms of regulation that largely implicate epigenetic factors, such as, DNA methylation, histone post-translational modifications, and non-coding RNAs (ncRNAs). Through these molecular pathways, some of the behavioral phenotypes associated with life experiences can be stably transmitted to descendants, sometimes across several generations. Rodent studies indicate that parental stressful and traumatic experiences can lead to behavioral despair, risk-taking behaviors, altered sociability and atypical responses to stressful stimuli in the offspring, whereas parental environmental enrichment has been associated with improved cognition and stress resilience in the offspring. Similar observations have been made in humans; children and grandchildren of genocide survivors show increased psychopathology and emotional disturbances. At the molecular level, changes in germline ncRNAs have been identified as likely vectors of transmission in rodents. The mechanisms linking behavioral stimuli to the germline, and factors responsible for these changes and their persistence across generations remain, however, largely unidentified.

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traumatic stress often lead to not only depression, anxiety, hostility, antisocial behaviors and addiction but also metabolic dysregulation in adulthood [reviewed in Ref. 1]. In contrast, positive life experiences such as environmental enrichment are associated with improved cognition and resilience against depression [2]. These cognitive and behavioral effects involve intricate changes in gene expression in the brain through complex epigenetic mechanisms involving DNA methylation or hydroxymethylation, histone post-translational modifications (PTMs), and non-coding RNAs (ncRNAs) such as micro-RNAs (miRNAs/miRs) [3]. Emerging evidence suggests that changes in behavior and cognition induced by environmental insults or life experiences can also be transmitted across generations, even if the offspring is not exposed to the initial causative event. Although behavioral phenotypes in the offspring can be transmitted by social and maternal factors during rearing or through passage of body fluids during delivery and/or lactation [reviewed in Refs. 3, 4], true biological inheritance of acquired behavioral phenotypes involves the germline, which is the focus of this review.

## Inter- and transgenerational epigenetic inheritance

The notion of inheritance of environmentally-induced traits dates back to the 19th century, when Lamarck proposed that environmental exposures could lead to biological adaptations in animals that are then passed onto the subsequent generations [5]. The past two decades have witnessed a rapid accumulation of studies in both rodents and humans that support a germline-dependent transmission of acquired traits to the next generation(s) implicating some epigenetic mechanisms [Reviewed in Ref. 3]. The environmental exposures that have been shown to be associated with inherited phenotypes include, 1) exposure to toxicants such as endocrine disruptors for example vinclozolin [6], 2) nutritional insult such as high-fat diet [7], 3) life experiences such as traumatic stress [8] or environmental enrichment [9], or parental diseases such as diabetes mellitus [10]. Exposure can be prenatal, early postnatal or in adulthood, and lead to inherited phenotypes across one or multiple generations. Transmission from a directly exposed parent to the offspring is typically called intergenerational epigenetic inheritance, while transmission across several generations is called transgenerational epigenetic inheritance (TEI). A hallmark feature of epigenetic inheritance is the presence of a specific epigenetic signature in the germline of the exposed

## Introduction

Life experiences, whether negative or positive, have a long-lasting impact on cognitive abilities and behavior in individuals. Adverse events in childhood such as

animal, which is generally accompanied by similar changes in somatic cells of the exposed animal and the offspring. Germline-dependence in TEI studies has been elegantly demonstrated using assisted reproduction technologies such as artificial insemination or *in vitro* fertilization (IVF), or transfer of molecular material such as RNA from the exposed sperm to fertilized control oocytes [reviewed in Ref. 3]. This review describes current evidence for epigenetic transmission (both inter and transgenerational) of both negative and positive behavioral phenotypes in rodents and humans, with an emphasis on groundbreaking and recent studies.

### Inter- and transgenerational epigenetic inheritance of negative behavioral traits

A growing body of evidence suggests that behaviors such as pro-depressive symptoms resulting from exposure to adverse environmental stimuli such as stressful events can be transmitted across generations. In experimental animals such as rodents, paradigms reproducing adverse conditions such as disrupted or impaired maternal care, maternal separation, fear conditioning, chronic stress or exposure to addictive substances have been used to demonstrate inter- and transgenerational transmission of behavioral phenotypes [reviewed in Ref. 4].

One of the most notable animal models is based on unpredictable maternal separation combined with unpredictable maternal stress (MSUS), an early postnatal paradigm of chronic trauma that leads to depressive-like symptoms, increased risk-taking, and antisocial behaviors up to the third or even the fourth generation [8,11,12]. In this model, hippocampus-dependent object recognition memory and synaptic plasticity are also impaired in exposed mice and their offspring [13]. These changes are accompanied by alterations in serotonergic circuits in the adult brain across generations [14], and by metabolic changes in many brain regions [15] and blood [8]. Interestingly in this model, transmission occurs through both fathers [11] and mothers [16], suggesting that both, male and female germlines are likely affected. Stress in adulthood in the form of disrupted social hierarchy also leads to anxiety and social deficits across three generations in mice [17]. Likewise, odor fear conditioning in adult males induces sensitivity to the same odor in their offspring and grand-offspring, an effect accompanied by neuroanatomical changes in the olfactory system [18].

Further to trauma or stress exposure, exposure to drugs of abuse can also cause inter-generational symptoms in experimental animals. The offspring of males addicted to cocaine have deficits in hippocampus-dependent memory and NMDA-dependent hippocampal plasticity even if never exposed to cocaine themselves. These deficits are rescued by administration of D-serine in the hippocampus, suggesting that the effects of the drug are reversible and have not damaged neuronal circuits

permanently [19]. Further, alcohol exposure for 4 weeks in female rats before conception causes an exaggerated stress hormone response after an immune challenge in their offspring and dysregulates the expression of several stress-related genes in various brain regions [20]. Exposure of males to long-term intermittent ethanol vapor before conception decreases preference to ethanol in their offspring and increases their sensitivity to the anxiolytic effects of ethanol, which correlates with higher expression of brain-derived neurotrophic factor (BDNF) in the ventral tegmental area (VTA) [21].

Transgenerational transmission of behavioral phenotypes can also be observed with certain exposures during embryogenesis, thus, excluding any social factors after birth but rather an implication of epigenetic mechanisms. Thus, *in utero* exposure to valproic acid causes autism-like behaviors in mice across three generations [22]. Similarly, *in utero* exposure to immune activation induces behavioral despair, altered sociability, and increased fear across several generations in mice [23].

Epidemiological evidence for possible inter- or transgenerational epigenetic inheritance of behavioral phenotypes in humans has steadily accumulated over the years [reviewed in Ref. 4]. However, it is only recently that some molecular analyses have started to be conducted to identify whether epigenetic factors are involved. The germline-dependence of such inheritance is not established in humans but initial results indicate that it is likely the case. There is evidence for increased psychopathology in the offspring and grand-offspring of holocaust survivors [24–26] and recently, changes in DNA methylation in the gene coding for FK506 binding protein 5 (*FKBP5*) were identified as a possible epigenetic signature of symptoms [27]. Similarly, genocide exposure in Cambodia and Rwanda has been linked to increased hyper-arousal and anxiety and depression in descendants, respectively [28,29]. The offspring of veterans of Serbian-Bosnian war with PTSD also have neurodevelopmental delays and emotional deficits [30] while daughters of Finnish women evacuated during the Second World War have increased risk of psychiatric hospitalization [31]. A very recent study examined the sperm of men with adverse childhood experiences and showed alterations in some miRNAs that are similar to that observed in mice exposed to stress and their offspring [32]. These results provide encouraging evidence that like in experimental animals, the germline may be implicated in epigenetic inheritance in humans.

Thus, evidence for inter- or transgenerational inheritance of behavioral phenotypes after negative stimuli is concrete in both rodents (Table 1) and humans (Table 2), and some molecular contributors of such inheritance have started to be delineated in rodents (Table 1).

**Table 1****Notable studies on intergenerational and transgenerational epigenetic inheritance of behavioral phenotypes in rodents in the last five years**

Experimental exposure (species)	Timing of the exposure	Associated inherited behavioral phenotype	Associated somatic/germ-line epigenetic changes	Number of generations affected	Reference(s)
Daily unpredictable separation of pups from the mother combined with unpredictable maternal stress (mice)	Early post-natal	Increased depressive, antisocial, and risk-taking behaviors. Impaired object recognition memory. Improved goal-directed behaviors. Enhanced anxiety and social deficits and elevated serum corticosterone	Altered ncRNA expression in sperm, hippocampus and serum of exposed mice and hippocampus and serum of the offspring. Altered DNA methylation in the brain and sperm of exposed mice and their offspring	3	Gapp <i>et al.</i> [8,37] Bohacek and Mansuy [3], Bohacek <i>et al.</i> [13] Gapp <i>et al.</i> [36*] Razoux <i>et al.</i> [14] Gapp <i>et al.</i> [16]
Chronic social instability and disruption of social hierarchy (mice)	Late post-natal	Increased sensitivity to conditioned odor	CpG hypomethylation in the <i>Olf151</i> gene in sperm and olfactory cortex	3	Saavedra-Rodríguez and Feig [17]
Pre-conception odor fear conditioning (mice)	Late post-natal	Reduced HPA-axis reactivity	Altered expression of nine different miRNAs in sperm	2	Dias and Ressler [18]
Pre-conception stress (mice)	Late post-natal or adulthood	Increased anxiety and reduced fear extinction	Increased miR-98, miR-144 and miR-190b in sperm	3	Rodgers <i>et al.</i> [46]
Pre-conception corticosterone treatment for four weeks (mice)	Late post-natal	Decreased ethanol preference and increased ethanol sensitivity in the male offspring	Increased BDNF expression in VTA	2	Yeshurun <i>et al.</i> [9,42]
Chronic intermittent exposure to ethanol vapor (mice)	Late post-natal	Gender-specific increase in latency to immobility in the forced swim test	Increased expression of miR-212/132 in sperm and hippocampus of enriched mice	2	Rompala <i>et al.</i> [21]
4 weeks of EE (mice)	Late post-natal	Increased synaptic plasticity and improved memory in the offspring		2	Yeshurun <i>et al.</i> [9,42]
10 weeks of EE (mice)	Late post-natal			2	Benito <i>et al.</i> [34*]

**Inter- and transgenerational epigenetic inheritance of positive behavioral traits**

The possibility that favorable environmental conditions can have benefits that can be transmitted across generations has recently emerged as a topic of interest. Studies in rodents have employed environmental enrichment (EE) as a paradigm to provide positive conditions to parents and assessed the cognitive and behavioral effects

in the progeny (Table 1). Exposure to 2-week EE starting on postnatal day 15 in male mice increases long-term potentiation and memory abilities in the offspring [33]. Likewise, 4-week EE before conception in male mice induces a form of resistance to depression in the offspring and lowers cortisol response to acute stress [9]. The effects of paternal EE on synaptic plasticity and cognition in the offspring have been proposed to implicate RNA in

**Table 2****Notable studies of intergenerational epigenetic inheritance of behavioral phenotypes in humans during the last five years**

Exposure	Associated inherited behavioral phenotype	Reference
Holocaust survivors	Increased psychopathology, increased susceptibility to PTSD, and low baseline cortisol levels in the offspring along with reduced methylation of <i>FKBP5</i>	Yehuda <i>et al.</i> [27*]
Genocide exposure in Cambodia	Increased anxiety in the offspring	Field <i>et al.</i> [28]
Genocide exposure in Rwanda	Increased anxiety and depression in the offspring	Rieder and Elbert [29]
Evacuation during the second world war in Finland	Increase psychiatric hospitalizations in women whose mothers were evacuated	Santavirta <i>et al.</i> [31]

germ cells [34<sup>•</sup>]. EE has also been shown to allow the correction of LTP and fear memory deficits in the offspring of Ras-GRF knock out mice [33] and to reverse the co-morbid anxiety in the offspring of a genetic model of absence seizures in mice [35]. It can as well rescue the negative effects of traumatic conditions in male mice and prevent their transmission to the offspring. Thus, exposure of fathers to EE shortly after chronic trauma reverses the altered coping behaviors and preserves their progeny from being affected. The behavioral rescue is accompanied by a correction of alterations in DNA methylation in the glucocorticoid receptor gene in the sperm of fathers and the hippocampus of the offspring, suggesting the implication of epigenetic mechanisms [36<sup>•</sup>].

An important consideration in this respect is that negative and positive behavioral traits resulting from exposure are not mutually exclusive. Early life trauma in the MSUS model has been shown to be also associated with increased behavioral flexibility and improved goal-directed behaviors in the offspring [37]. Further in this model, while *N*-acetyl aspartate is lower in the adult brain of exposed animals consistent with neuronal dysfunction, it is not decreased after a stress challenge as expected in the brain of the offspring [16], suggesting a form of coping. Transmission of such positive traits after a traumatic experience may confer an advantage to the offspring in challenging conditions and may have important evolutionary implications [38].

### Mechanisms underlying the transgenerational inheritance of behavioral phenotypes

Recent research in rodents has provided evidence that TEI involves the germline but two important questions deserve particular attention: 1) How can the effects of purely emotional/psychological stimuli initiated in the brain reach the germline, 2) What are the vectors of transmission of these effects? Among the most plausible candidates, circulating factors are the most likely to be able to carry stimuli initiated centrally to germ cells. Hormones, cytokines, or circulating DNA or RNA may be such factors since they can be affected by life experiences and behavioral stimuli [39–41]. Immune-activation that can potentially alter the cytokine milieu [23], and hormonal treatments [42] have been shown to alter the germline epigenome. The maturing sperm depends on the acquisition of circulating ncRNAs and proteins from extracellular vesicles called epididymosomes during its passage in the epididymis [43]. Therefore, changes in these factors could bridge the brain to the germ-line and contribute to the transmission of a molecular signature of exposure to the offspring.

Changes in the germline epigenome are currently considered as the most likely means to mediate TEI. Among the three most studied epigenetic factors, DNA methylation, histone PTMs and ncRNAs, ncRNAs are the best

described candidates today. Several TEI studies have shown that their level, in particular of miRNAs, is altered in the sperm after exposure [8,44<sup>••</sup>,45,46]. The causal implication of RNA from male germ cells in epigenetic inheritance has been examined in ‘proof-of-concept’ experiments. One of the pioneering studies showed that injecting RNA extracted from the sperm of males exposed to traumatic stress when pups, into fertilized control oocytes recapitulated trauma-induced behavioral and metabolic symptoms in the resulting progeny [8], providing a causal link between sperm RNA and symptoms across generations. Injection of nine miRNAs found to be altered by adult stress in mice and associated with decreased blood corticosterone were also reported to reproduce the effect on corticosterone [47]. Likewise, injection of sperm RNA from males exposed to 10-week EE could recapitulate enhanced LTP and cognitive functions in the resulting offspring, and the effects were prevented when inhibitors of miR-212/132 were co-injected [34<sup>•</sup>], suggesting the implication of miR-212/132.

### Conclusions and outlook

The past few years have witnessed a rapid accumulation of evidence supporting the idea that life experiences and environmental exposures can lead to specific behavioral phenotypes that can be transmitted across generations (Tables 1 and 2). The germline-dependence and the involvement of epigenetic factors in this form of inheritance have been documented and some of the vectors of transmission have been identified to involve RNA in rodent studies. A number of questions remain, however, unanswered. First, while potential vectors of transmission have been proposed, the connection between the initial behavioral/environmental stimulus and the epigenetic signature in germ cells remains unknown. Second, it is not clear how the phenotypes associated with transgenerational inheritance are further passed on beyond the first generation. Third, more direct causal proof still needs to be provided and ‘reversal’ studies showing a correction of epigenetic alterations like in Gapp *et al.* [36<sup>•</sup>] or in Benito *et al.* [34<sup>•</sup>] are needed. Reversal studies could employ parental and/or offspring environmental manipulations known to have transgenerational effects such as EE [9,34<sup>•</sup>,36<sup>•</sup>] or exercise [48], or molecular manipulations in germ cells such as ncRNA silencing [34<sup>•</sup>] or epigenome editing through CRISPR-dCas [49]. Importantly, different mechanisms may be responsible for the transmission of phenotypes depending on the environmental stimuli, the time window and severity of exposure, and the time of analyses (whether shortly or long after exposure). Different mechanisms may also operate in females and males, and systematic analyses in both genders need to be considered. Finally, an important next step will be to assess epigenetic factors in human in different body fluids such as blood, saliva, sperm or milk as initiated for some conditions and relate them to direct or ancestral exposures [4,50]. Such studies shall provide



new knowledge about the etiology and mechanisms of expression of environmental pathologies and will help explain the ‘missing heredity’ of neuropsychiatric disorders. They may have wide-ranging implications for preventive and personalized medicine in the future [4].

## Conflict of interest statement

Nothing declared.

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